Chronic Effects of Pesticide Mixtures on the Survival, Development, and Metamorphosis of the Iberian Painted Frog

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Background

- **Amphibians** are experiencing **severe population declines**, often in habitats highly vulnerable to pesticide runoff from **nearby agricultural areas** [1].
- **Regulatory agencies do not mandate amphibian-specific toxicity tests**, and surrogate vertebrate models often overlook their specific vulnerabilities [2].
- Species **breeding in ephemeral wetlands** face **risks from delayed metamorphosis and drying habitats**, making **development time critical** [3].

Iberian painted frog

Objective:

Assess the lethal and sublethal effects of **chronic larval exposure** to the **herbicide 2,4-D**, the **insecticide flupyradifurone**, and their mixture in the **Iberian Painted Frog** (*Discoglossus galganoi*), a species inhabiting ephemeral wetlands.

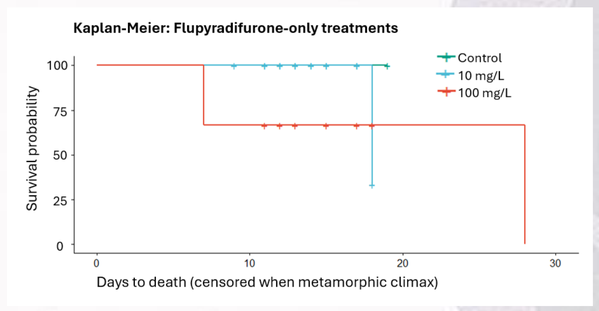
Experimental design

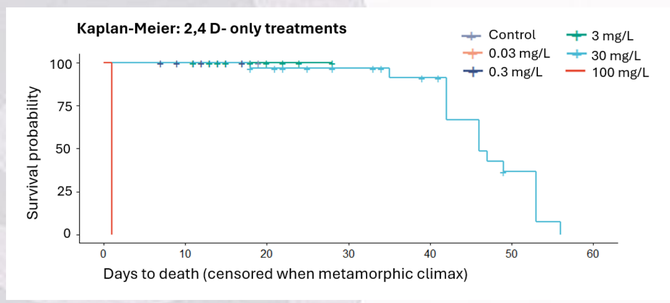
Treatments		Flupyradifurone 0	Flupyradifurone 10 mg/L	Flupyradifurone 100 mg/L
2,4-D	0	✓	✓	✓
	0.03 mg/L	✓		
	0.3 mg/L	✓	✓	✓
	3 mg/L	✓		
	30 mg/L	✓	✓	✓
	100 mg/L	✓		

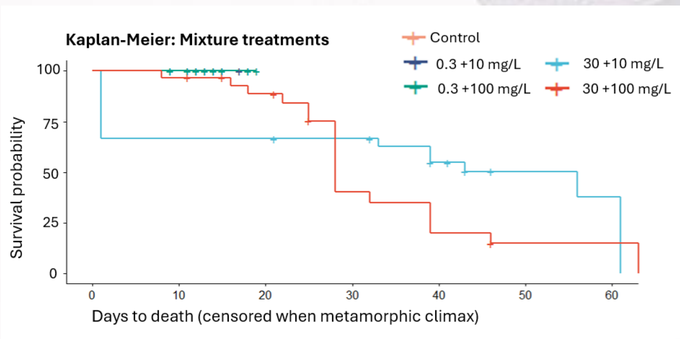
Pesticide exposure began at **free-swimming larval stages**, with each treatment replicated **three times** using **10 tadpoles per aquarium**.

Endpoints included survival, growth, time to metamorphosis, metamorphic success (% completion), and body condition index (BCI = [mass/Snout-Vent Length^3] × 100).
Statistical analyses included Kaplan–Meier survival curves (censored at metamorphic climax) and GLMMs for survival and time to both metamorphic climax and completion.

Survival during larval stages





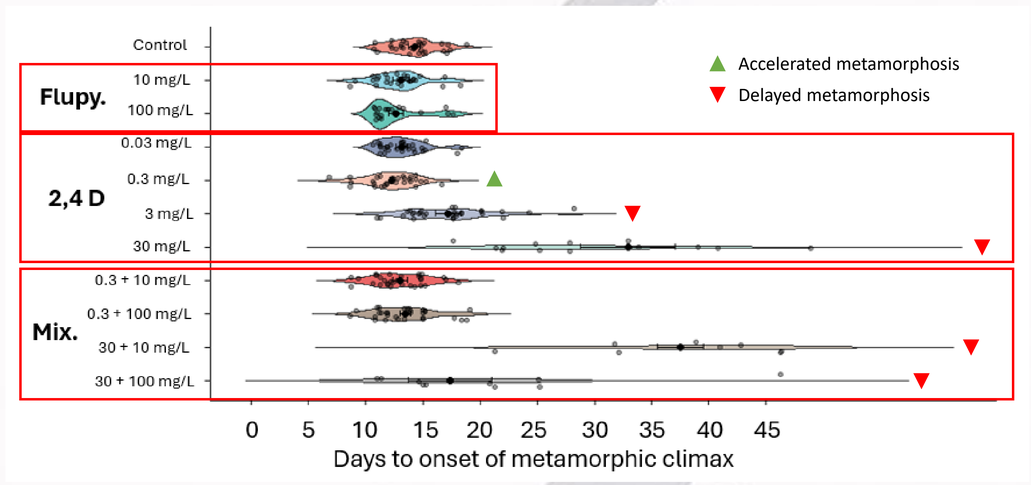


High-dose and **mixture exposures** led to significantly **reduced survival**.

The **100 mg/L 2,4-D group showed 100% mortality within 24 h**.

Significant survival reductions occurred at 100 mg/L flupyradifurone group ($p = 0.0302$) and in the 30 + 10 mg/L mixture group ($p = 0.0302$).

Time to metamorphic climax



High concentrations of 2,4-D (≥ 3 mg/L), especially when **combined with flupyradifurone**, caused **pronounced delays** in developmental progression (all $p < 0.0001$).

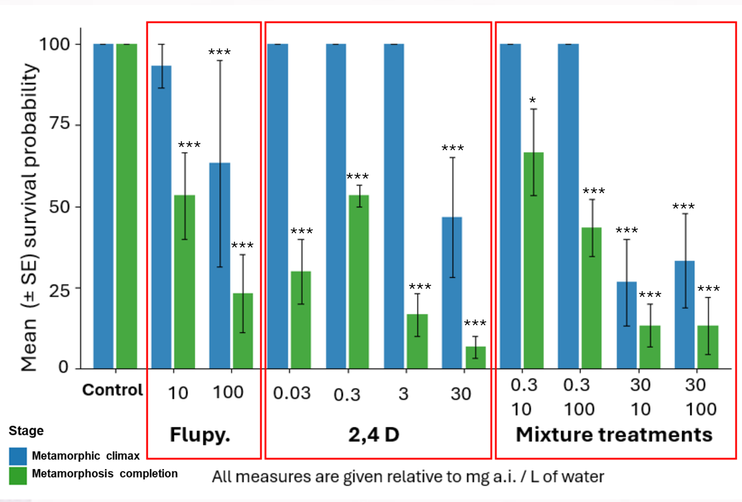
Some **lower-dose exposures**—notably 0.3 mg/L 2,4-D—were associated with **accelerated metamorphosis** ($p = 0.0256$), while others showed no significant effect.

- **Control group**: 14.07 ± 0.41 days to metamorphic climax.
- **30 + 10 mg/L mixture group**: 43.50 ± 1.50 days **(+29.4 days vs. control).**
- **0.3 mg/L 2,4-D group**: ▸ 11.38 ± 0.65 days **(–2.7 days vs. control).**

Survival during metamorphosis completion

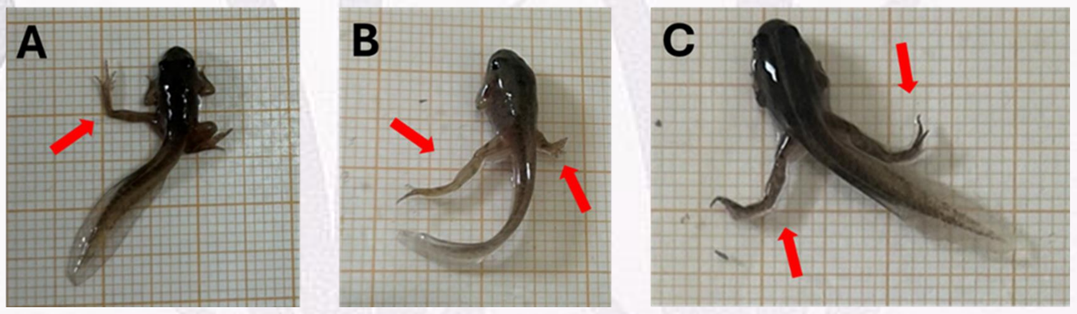
2,4-D consistently **reduced survival** in a **dose-dependent manner** at both metamorphic climax and metamorphosis completion ($p < 0.001$).

Flupyradifurone-only treatments had no significant effect on survival at metamorphic climax ($p = 0.128$), but **significantly reduced survival** at **metamorphosis completion** in pairwise comparisons versus the control group (all $p < 0.001$).



All treatments except controls **exhibited mortality at metamorphosis completion, highlighting developmental vulnerability even at lower concentrations or combinations**.

Developmental malformations



Limb malformations were observed, especially in the 3 mg/L 2,4-D treatment: 66.7% of survivors to metamorphic climax were affected.
Malformations **significantly reduced survival** ($p < 0.001$) → ~87% lower chance of surviving to metamorphosis completion.
Abnormalities included hind limb hypoplasia, digit deformities and joint misalignments.
Suggests **disruption of musculoskeletal development.**

Conclusions

- **2,4-D reduced survival** in a **dose-dependent manner** and induced strong developmental disruptions, while **flupyradifurone**—though subtler in early stages—also contributed to **significant mortality during late metamorphosis**.
- Mixtures showed **non-additive effects on timing**.
- **Delays and malformations often occurred without early mortality**, highlighting the importance of chronic and developmental endpoints.

About me:

PhD candidate (defense expected early 2026)

Motivated to pursue postdoctoral research in ecotoxicology, focusing on how environmental stressors affect amphibian life-history and ecophysiology.

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[1] **EFSA (2018)**. *Scientific opinion on the state of the science on pesticide risk assessment for amphibians and reptiles*. EFSA Journal, 16(2): e05125.
[2] **Ortiz-Santaliestra, M. E., Maia, J. P., Egea-Serrano, A., & Lopes, I. (2018)**. *Validity of fish, birds, and mammals as surrogates for amphibians in pesticide toxicity assessment*. Ecotoxicology, 27(7), 819–833.
[3] **Préau, C., Bertrand, R., Brühl, C. A., & Miaud, C. (2022)**. *Can pesticide-driven delays in metamorphosis jeopardize the persistence of amphibian populations?* Environmental Pollution, 295, 118696.